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TWO TYPES OF GLUTAMATE RECEPTORS DIFFERENTIALLY EXCITE AMACRINE CELLS IN THE TIGER SALAMANDER RETINA

BY DONALD B. DIXON AND DAVID R. COPENHAGEN

From the Departments of Ophthalmology and Physiology, University of California, San Francisco, CA 94143-0730, USA

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SUMMARY

1. Excitatory inputs to amacrine cells in the salamander retinal slice preparation were examined using whole-cell patch pipette voltage-clamp techniques. In strychnine (500 nM) and bicuculline (100 μM), two types of amacrine cell were easily distinguished by their light-evoked excitatory responses: *transient* and *sustained*.

2. In *transient* amacrine cells the current–voltage (*I–V*) relation for the peak light-evoked current was non-linear with a negative slope region between −50 and −70 mV. Responses reversed near +10 mV and were prolonged at more positive holding potentials.

3. In DL-2-amino-phosphonoheptanoate (AP7, 30 μM), a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist, both the negatively sloped region of the light *I–V* relation and the prolongation of the response at positive potentials were eliminated. In 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 2 μM), a selective non-NMDA receptor antagonist, light-evoked currents at the most hyperpolarized holding potentials were eliminated. At potentials positive to −85 mV the light-evoked currents lacked a fast onset. The light *I–V* relation in CNQX had a negative slope region between −35 and −80 mV.

4. With synaptic transmission blocked, kainate evoked responses in *transient* cells with a resultant *I–V* relation that was nearly linear, whereas glutamate and NMDA elicited responses with non-linear *I–V* relations.

5. Light-evoked currents in *sustained* amacrine cells had a nearly linear *I–V* relation and reversed near +10 mV. AP7 at a concentration of 30 μM did not affect the light-evoked currents in *sustained* cells, but 2 μM-CNQX eliminated all light-evoked currents in these cells.

6. With synaptic transmission blocked, *sustained* amacrine cells responded only to glutamate and kainate, not NMDA. The resultant *I–V* relations were linear.

7. We conclude that the light-evoked responses of *transient* amacrine cells are mediated by concomitant activation of both non-NMDA and NMDA receptors whereas the responses of *sustained* amacrine cells are mediated only by non-NMDA receptors. Furthermore, these data provide supportive evidence that the primary light-evoked excitatory neurotransmitter activating amacrine cells is glutamate.

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INTRODUCTION

Glutamate is a prominent excitatory neurotransmitter in the brain and retina. Several classes of glutamate receptor have been defined, using pharmacological, molecular biological, and electrophysiological techniques (Mayer & Westbrook, 1987*a*; Trussell, Thio, Zorumski & Fischbach, 1988; Sommer, Keinanen, Verdoorn, Wisden, Burnashev, Herb, Kohler, Takagi, Sakmann & Seeburg, 1990). The distribution and density of glutamate receptors varies throughout the central nervous system (Boulter, Hallmann, O'Shea-Greenfield, Hartley, Deneris, Maron & Heinemann, 1990; Nielsen, Drejer, Cha, Young & Honore, 1990). The variation in subtype and differential distribution of these receptors suggests that glutamate may have several distinct actions on nerve cells. In addition to serving as a chemical relay of fast excitation at synapses, glutamate may perform modulatory or long-term functions by interacting with different receptor subtypes. Two well-studied glutamate receptor subtypes are the *N*-methyl-D-aspartate (NMDA) receptor and the quisqualate/kainate receptor; the latter is commonly referred to as the non-NMDA receptor. Non-NMDA receptor channels respond to agonists rapidly and also can inactivate rapidly. They are voltage independent and are permeable to monovalent cations predominantly (Randle, Vernier, Garrigues & Brault, 1988). In contrast, NMDA receptor channels display much slower kinetics, both at the onset and decay of the response (Jahr & Stevens, 1990). These NMDA channels are voltage dependent (Mayer, Westbrook & Guthrie, 1984; Forsythe & Westbrook, 1988) and are permeable to most monovalent cations and calcium, and can be involved in long-term modifications of synaptic efficacy (Collingridge, Kehl & McLennan, 1983). At synapses from most, but not all glutamatergic cells (Perkel, Hestrin, Sah & Nicoll, 1990), glutamate activates a combination of non-NMDA and NMDA receptors. It is evident that the kinetics, voltage dependence and possibilities for long-term modifications of glutamatergic inputs will depend on the mix of the two receptor types at any given synapse.

In non-mammalian retinae, the bipolar cells, which are glutamatergic (Ehinger, Ottersen, Storm-Mathisen & Dowling, 1988; Marc, Liu, Kalloniatis, Raiguel & Van Haesendonck, 1990), provide the predominant excitatory input to both amacrine and ganglion cells. Amacrine cells, which stain heavily for GABA and glycine (Yang & Yazulla, 1989*a*, *b*) provide the main inhibitory inputs to ganglion cells and also feedback to bipolar cell terminals (Wong-Riley, 1974). Mittman, Taylor & Copenhagen (1990) demonstrated that the excitatory light responses of 'on' and 'on-off' ganglion cells result from concomitant activation of both non-NMDA and NMDA receptors. Previous authors (Coleman & Miller, 1988; Massey & Miller, 1990) have shown that retinal ganglion and amacrine cells respond to both non-NMDA and NMDA agonists. However, their general conclusions were that NMDA receptors might play minor roles in the on pathway (Lukasiewicz & McReynolds, 1985), or are extrasynaptic and not important at all in the generation of the light response in either ganglion or amacrine cells. Because the study by Mittman *et al.* (1990) did demonstrate a prominent NMDA component of the light response in ganglion cells, it seemed prudent to re-evaluate the nature of the glutamate receptors responsible for amacrine cell excitation. The importance of defining the mix of non-NMDA- and

NMDA-mediated inputs to amacrine cells is to determine the kinetics, voltage dependency and possibilities for use-dependent modification of the excitatory inputs to these cells. These characteristics, in turn, will have a major bearing on the nature of the inhibitory inputs from these cells onto ganglion cells and back onto bipolar cells.

We have used patch-clamp techniques combined with a variety of pharmacological manipulations to investigate the nature of light-evoked excitatory responses in amacrine cells. We have found that both non-NMDA and NMDA receptors play a significant role in the light response of transient amacrine cells, whereas NMDA receptors seem absent from sustained amacrine cells, and only non-NMDA receptors participate in their light response. These results raise the possibility that different glutamate pathways serve separate visual functions.

METHODS

Neotenic tiger salamanders, *Ambystoma tigrinum*, were obtained from Lowrance Waterdog Farm and maintained at 4 °C with a 12 h light–dark cycle. Prior to experimentation animals were dark adapted for at least 1 h. All subsequent procedures were done under dim, 700 m light. Animals were decapitated and double pithed. Eyes were enucleated and retinal slices, 150 μm thick, were prepared as described by Werblin (1978) and Mittman *et al.* (1990). No enzyme treatments were used on the preparation.

The superfusion solution consisted of (mM): NaCl 104, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1, glucose 5, and HEPES 5, buffered to a pH of 7·6 with NaOH. Strychnine chloride (500 nM) and bicuculline methobromide (100 μM) were added directly to the superfusion solution to eliminate inhibitory synaptic inputs. All the above chemicals were reagent grade from Sigma, St Louis, MO, USA. The superfusion rate was 1 ml min^{-1} and the chamber volume was 250 μl.

Cadmium (20 μM) or manganese (5 mM) (the latter for NMDA microperfusion) was added to the superfusion solution during the agonist microperfusion experiments (Figs 3 and 5) to eliminate synaptic transmission and thereby control for the possibility that the agonist effects we observed were not due to actions on the photoreceptor–bipolar cell synapses. Cadmium effectively blocked the light-evoked responses and was used for the kainate and glutamate experiments. However, cadmium was problematic in the NMDA experiments since it also blocks most of the NMDA current (Mayer & Westbrook, 1987*b*) so manganese was used instead. Manganese completely blocked light-evoked responses in amacrine cells and there was no evidence that NMDA overode the manganese block of neurotransmission. It was evident, however, that manganese did not always completely eliminate neurotransmission when kainate and glutamate were used as agonists. During the smooth course of the glutamate- or kainate-induced response, sharp transients were noted occasionally. These transients were similar in shape and time course to synaptically evoked transients. We speculate that because kainate and glutamate are potent agonists with many sites of action in the outer retina, the stimulatory effects of the high agonist concentrations used in our experiments may have been sufficient to occasionally override the manganese block of synaptic transmission. In contrast, NMDA has fewer binding sites in the outer retina making it less potent and less likely that its stimulatory effect would supercede the manganese block.

Agonists were microperfused via flow pipes made from polyethelene tubing pulled to an inside diameter of 100–120 μm. These flow pipes were attached to the water-immersion objective lens and were positioned approximately 200 μm from the selected cell. A series of control valves was used to select different agonists. Agonist concentration in the flow pipes was 250 μM and the perfusion time was 0·25 s.

The glutamate receptor antagonists DL-2-amino-phosphonoheptanoate (AP7) (Cambridge Research Biomedicals, Natick, MA, USA) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Tocris Neuramin, Essex) were mixed directly into the superfusion saline. Light responses were recorded first in control saline, then after 5 min of perfusion with the antagonist, and again after a 10 min wash-out period.

Patch pipettes were pulled from Corning 8161 glass (Garner Glass, Claremont, CA, USA) and the

taper was coated with Sylgard (Dow Corning, Midland, MI, USA) to reduce electrode capacitance. Tips were approximately 1 μm in diameter. The pipette solution consisted of (in mM): CsF 84, NaCl 3·4, $MgCl_2$ 0·4, $CaCl_2$ 0·4, EGTA 11, HEPES 10, adjusted to pH 7·6 with CsOH. ATP (1 mM), and GTP (0·1 mM) were used in some experiments. We used EGTA to ensure a low intracellular calcium concentration since prolonged exposure to elevated intracellular calcium can be detrimental to the cell. For current-clamp recordings, KF (84 mM) was used in the pipette solution. Caesium was used as the main cation in most experiments to eliminate potassium currents which make voltage steps to potentials above −20 mV difficult to maintain. Fluoride was used as the main anion because it increased the likelihood of high quality patch seals. A caesium gluconate (84 mM) solution was used in some voltage-clamp experiments only as an alternative to caesium fluoride. No differences were noted between experiments done with fluoride or gluconate as the predominate anion.

An Axopatch 1B amplifier (Axon Instruments, Foster City, CA, USA) was utilized for voltage- and current-clamp recordings. The reference electrode was connected to the bath via a 3 M-KCl agar bridge and a Ag–AgCl pellet. Signals were filtered at 2 kHz and digitized. Clamp voltages were corrected for the liquid junction potential (−6 mV). Voltage clamp steps away from a holding potential of −81 mV were applied 400 ms before the light onset and terminated 2500 ms after the light stimulus was extinguished. An interval of 400 ms between the onset of the voltage clamp step and the light stimulus was sufficient to allow the voltage-dependent, time varying currents to reach steady state. Light stimuli were produced from a tungsten–quartz–iodine lamp and were filtered with several neutral density filters and a 520 nm interference filter. The intensity of the stimulus light ranged from 5×10^{16} to 5×10^{17} photons m^{-1} s^{-1}. These stimulus conditions would excite a mixed cone population of photoreceptors. Hoffman contrast optics were used to visualize the preparation during pipette placement and seal formation.

Cell identification

Recordings were done on cells at the distal edge of the inner nuclear layer where the cell bodies of most amacrine cells lie. Identification of cells as amacrines was based on the characteristics of the light response and the voltage-activated currents. Briefly, sustained amacrine cells could be distinguished from bipolar cells because the bipolar cell light response had a larger, less noisy, sustained current with a distinct overshoot at light off (data not shown). Bipolar cells also lack a voltage-activated sodium current (Kaneko & Tachibana, 1985) which is prominent in amacrine cells (Barnes & Werblin, 1986). Light-evoked current responses in interplexiform cells are typically slower and larger than those in sustained amacrine cells, and current pulses produce repetitive action potentials (data not shown; Maguire, Lukasiewicz & Werblin, 1990). 'On' sustained amacrine cells were distinguished from 'on' sustained ganglion cells by their more nearly linear *I–V* relations. If some displaced ganglion cells of the on type were recorded in this study they exhibited the phenotypical characteristics of amacrine cells and not those of the on ganglion cells recorded in the ganglion cells layer (Mittman *et al.* 1990).

Distinguishing transient amacrine cells from displaced transient ganglion cells was problematic. The few transient cells studied with KF in the electrode (necessary for current-clamp recordings) gave only a single spike at light on and off. This is characteristic of amacrine cells (Barnes & Werblin, 1986) and is in contrast to transient ganglion cells which fire several spikes at light on and off under the same recording conditions (Mittman *et al.* 1990). Most of our experiments were done with CsF which makes current-clamp recordings impractical and makes it difficult to discriminate between the two cell types. However, it is highly unlikely that displaced ganglion cells represented a significant proportion of the more than ninety transient cells studied, since displaced ganglion cells account for less than 5% of cells at the distal edge of the inner nuclear layer in the retina of a closely related species of salamander (Ball & Dickson, 1983).

Space clamp considerations

One must consider the prospect that the long amacrine cell processes were inadequately space clamped in these experiments. Although this cannot be ruled out unequivocally, several findings suggest that the processes are electrotonically close and could be satisfactorily space clamped. In all the experiments, the light- and agonist-induced responses could be taken beyond their reversal potential, consistent with satisfactory voltage control. Furthermore, there was never any evidence of action potentials during voltage-clamp pulses which would have indicated that active membrane conductances escaped voltage-clamp control. Consistent with the idea that conductance changes generated in the tips of dendrites can be recorded in the soma, or conversely, that these distal tips

could be voltage clamped from the soma, is a recent report by Bloomfield (1991) showing that the diameters of light-evoked receptive fields closely matched the dimensions of the dendritic arbours of amacrine cells.

RESULTS

Transient amacrine cells

Light responses of transient amacrine cells show a voltage dependence

Recordings were made from amacrine cell somata located at the proximal edge of the inner nuclear layer. The patch pipettes were visually guided to somata lying on the upper surface of the slice. Figure 1*A* shows the light response recorded in an on-off amacrine cell at different voltage-clamp holding potentials. Typical of transient cells, this cell responded with a brief current which began 100–150 ms after a light stimulus was turned on, and then a second transient which began about 100–150 ms after the light stimulus was extinguished. Similar to other transient amacrine cells in this study, the light-evoked currents in this cell reversed around +10 mV. The responses at more positive holding potentials had a longer latency and more prolonged time course. The oscillations seen in the −51 mV trace were not always present. These may be analogous to the oscillatory potentials noted in the electro-retinogram representing synaptic activity in the inner plexiform layer. Since their incidence was rare in our experiments, they were not studied further. The responses at the onset and termination of the light were of comparable amplitude in the cell shown in Fig. 1*A*. There was considerable variation in the sizes of the on and off transients amongst the on-off amacrine cells. Off transients were generally more prominent with the long duration light stimuli we used (> 1 s), hence these responses make up the bulk of the data for transient cells. When on responses were present for the duration of the experiment, they were included in the analysis. The latencies of single responses from individual on-off amacrine cells could vary over a range of about 80 ms from the mean. It was our impression that this flash-to-flash latency variation was more pronounced in amacrine than ganglion cells. The reasons for this variation were not examined in this study.

Figure 1*B* shows the averaged light-evoked peak *I–V* relation obtained from ten on-off amacrine cells. Current responses in each cell were first normalized to the maximum peak current for the individual cell, then values for all cells were averaged. The overall mean peak current amplitude in transient cells was −148·4 pA at −81 mV, and ranged between −112·8 and −226·4 pA. The light-evoked excitatory postsynaptic currents, measured at the peak of the response, reversed at about +10 mV and the slope of the *I–V* relation curve was nearly linear for holding potentials between −35 and +24 mV. From −35 to −85 mV the *I–V* relation was relatively flat indicating that the light-induced currents were similar in magnitude. Negative to −85 mV the light-evoked currents increased in size.

The prolongation of the light-evoked responses at potentials positive to −60 mV suggests that they are comprised of two or more components having different voltage dependencies. The non-linear peak *I–V* curve (Fig. 1*B*) is consistent with this idea. A comparison of the *I–V* relations measured at two time points during the light responses further supports this idea. Figure 1*C* shows, on an expanded time base, off responses from a transient amacrine cell. The dotted lines indicate the 'early' and

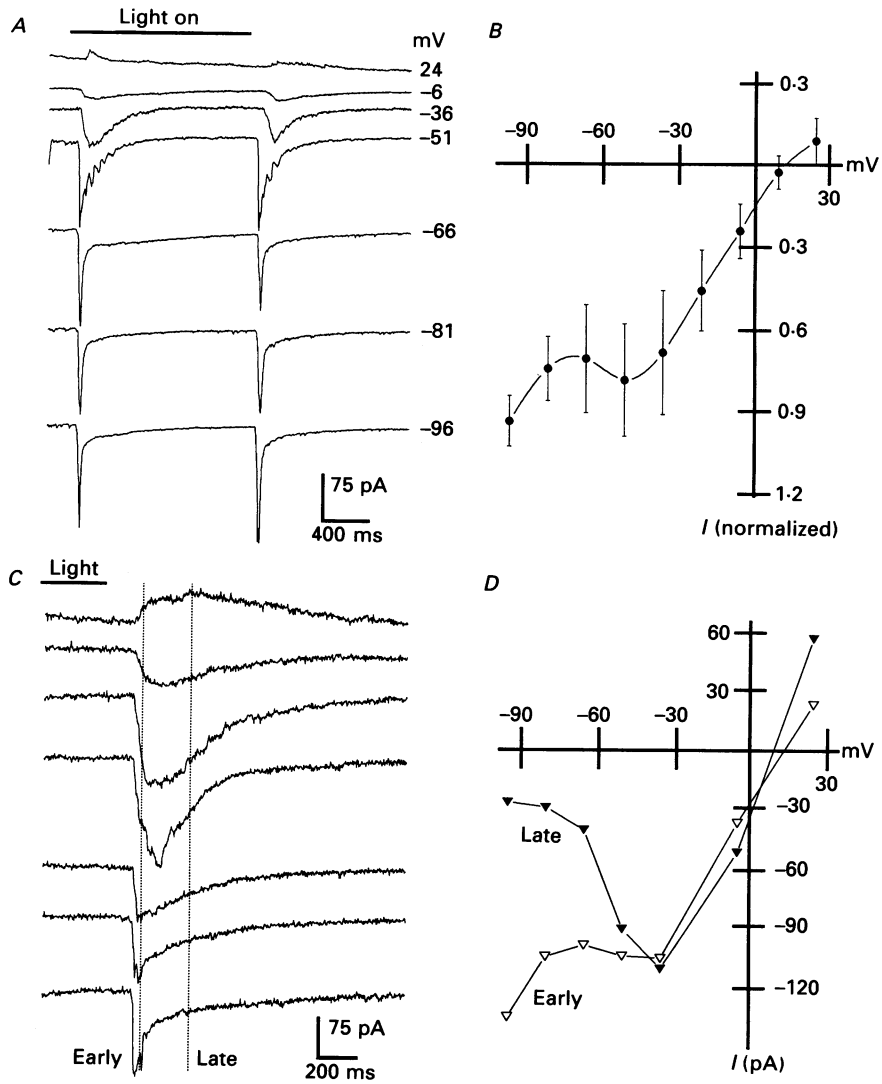

Fig. 1. Light-evoked responses in an on-off transient amacrine cell. *A*, tracings are the light-evoked currents obtained at those clamp potentials indicated to the right (*A*). The solid line at the top indicates the duration of light stimuli. *B*, average peak *I–V* curve of light responses in ten cells. The abscissa is membrane potential and the ordinate is normalized current (see text). Error bars are ±S.D. *C*, traces are of off responses recorded at the clamp potentials as listed to the right of *A*. Vertical dotted lines indicate the times (200 and 450 ms, respectively) after the light was extinguished at which the 'early' and 'late' *I–V* relations were calculated. *D*, *I–V* relations calculated (from tracings in *C*) at the early (▽) and late (▼) time points. The early *I–V* relation shows only a flattening between −35 and −65 mV, whereas the late *I–V* relation has a large negative slope region between −35 and −90 mV. Similar results were obtained in ten cells.

'late' time points at which the *I–V* relation was measured. Figure 1*D* shows the 'early' (▽) and 'late' (▼) *I–V* relations. The early *I–V* relation exhibits a slight flattening of the curve between −35 and −65 mV. In contrast, the late *I–V* relation

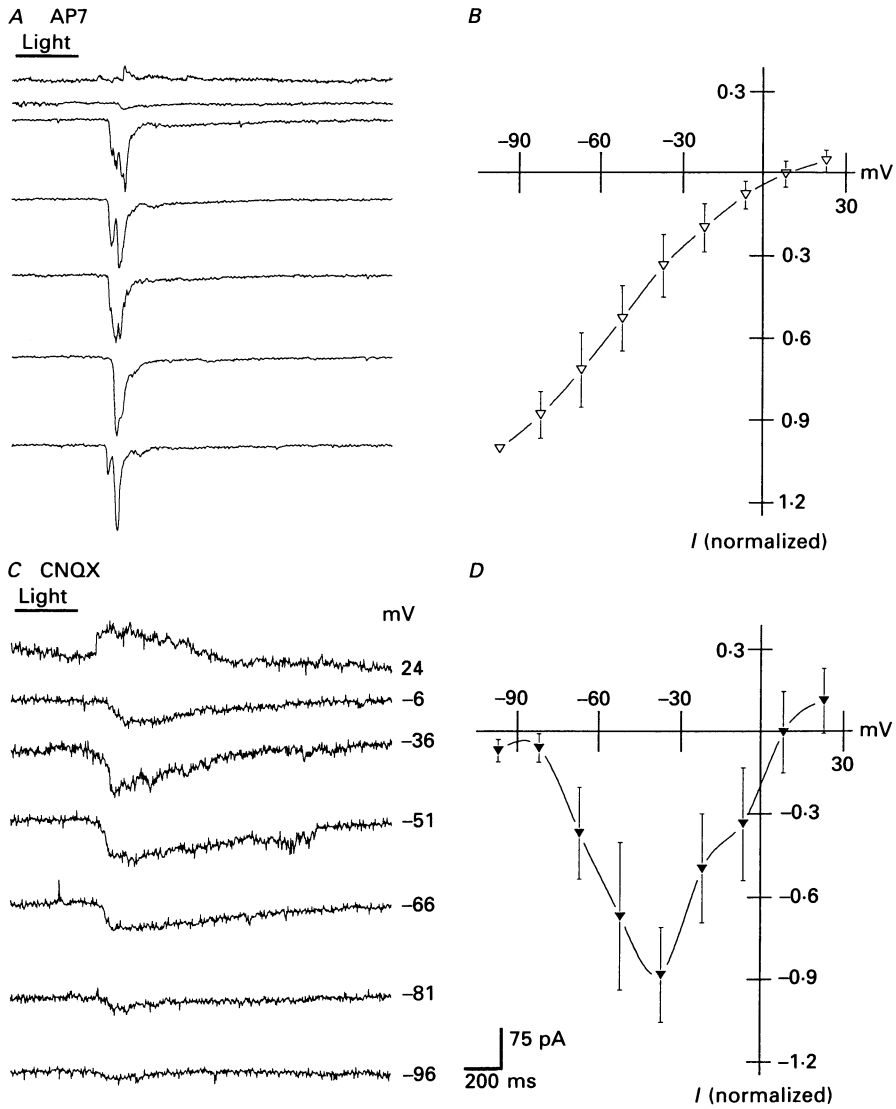

Fig. 2. Effects of glutamatergic antagonists on the light response of transient amacrines. The responses shown in *A* and *C* are from the same cell shown in Fig. 1*C*. *A*, off response recorded in AP7 (30 μM). Clamp potentials were identical to those shown in *C*. The prolonged portion of the response which is prominent at membrane potentials positive to -60 mV is absent. *B*, averaged, normalized *I–V* relation obtained from five cells recorded in AP7. The mean peak current at -96 mV was $-128{\cdot}3 \pm 56{\cdot}4$ pA. The curve is monotonic with no obvious flattening between -35 and -65 mV. Error bars indicate S.D. *C*, off response from the same cell recorded in CNQX (2 μM). The fast early portion of the response is eliminated by CNQX. *D*, averaged, normalized *I–V* relation obtained from five cells. The mean peak current at -36 mV was $-74{\cdot}3 \pm 21{\cdot}7$ pA. A pronounced negatively sloped region was evident between -35 and -80 mV.

has a prominent negative slope region between −35 and −95 mV. The shape of the late *I–V* relation reflects a light-evoked current which is strongly voltage dependent and which has a slow decay that is in evidence at potentials above −60 mV. The early portion of the transient current seems much less voltage dependent and has a faster decay. For example, compare traces at −96 and −36 mV in Fig. 1*A* and *C*. Further evidence for a multi-component light response was obtained from pharmacological experiments.

Non-NMDA and NMDA glutamate receptor antagonists differentially affect the light-evoked I–V relation

The prolongation of the response at depolarized potentials and the negative sloped *I–V* relation are characteristics of NMDA-mediated responses studied in other systems (Mayer & Westbrook, 1987*b*). We found that 30 μM-DL-2-amino-phosphonoheptanoate (AP7), an NMDA receptor antagonist (Olverman, Jones & Watkins, 1984), linearized the light-evoked *I–V* curve and eliminated the prolongation of the response in transient cells, consistent with a role for NMDA receptor activation in the light responses of these cells.

Figure 2*A* shows the off response recorded from the same on-off cell shown in Fig. 1*C*, but in 30 μM-AP7. The response still reversed near +10 mV and a plot of the peak current *versus* the holding potential shows that the relation is more linear than controls, clearly lacking a negative slope region (Fig. 2*B*). The increased response duration observed at potentials positive to −60 mV in control saline is also absent. This can easily be seen by comparing responses at −36 or −51 mV in control solution (Fig. 1*C*) and in AP7-containing solution (Fig. 2*A*). Cumulative results from five cells in control saline and five cells in AP7-containing saline held at −36 mV quantitatively substantiate these differences. In AP7, the response time to peak is 27·4 ± 11·9 ms (± S.D.) and the decay to 1/e of the peak amplitude is 46 ± 15·3 ms. This is in contrast to controls where time to peak is 43·2 ± 19·5 ms and the decay to 1/e of the peak amplitude is 302 ± 46·2 ms.

We also investigated the characteristics of the light response in 2 μM-6-cyano-7-nitroquinoxaline-2-3-dione (CNQX), a non-NMDA receptor antagonist (Yamada, Dubinsky & Rothman, 1989). Figure 2*C* illustrates the off response of the same cell shown in Figs 1*C* and 2*A* but recorded in CNQX. As the response develops at progressively more depolarized holding potentials, the response rise time was slow compared to controls and AP7 (Figs 1*A* and *C* and 2*A*) and the duration was much longer than seen in AP7 (Fig. 2*A*). Averaging the results from five amacrine cells in CNQX, we found that the response time to peak was 66·1 ± 11·3 ms and the response decay time to 1/e of the peak amplitude was 243 ± 57·2 ms at −36 mV. The pronounced negative slope in the peak *I–V* relation (Fig. 2*D*) for the cell shown in Fig. 2*C* is similar to the late *I–V* relation shown in Fig. 1*D*.

NMDA and non-NMDA glutamate receptor agonists elicit different responses

Cadmium (20 μM) is known to block synaptic inputs to ganglion cells (Mittman *et al.* 1990). Both non-NMDA and NMDA receptor agonists elicited currents in ganglion cells in the presence of cadmium, indicating that both classes of receptors are expressed on ganglion cells. We found that either 20 μM-cadmium or 5 mM-manganese blocked light responses in amacrine cells. While synaptic inputs were

eliminated, we applied non-NMDA and NMDA agonists to test for the presence of these receptors on transient amacrine cells. Glutamate ($n = 7$) generated responses which had an *I–V* relation similar to that evoked by light (Fig. 3*A* and *D*). A flattened region between −55 and −85 mV is apparent. During NMDA experiments we used manganese to block synaptic transmission because it permeates the NMDA receptor channel much better than cadmium (Mayer & Westbrook, 1987*b*). Under these conditions NMDA ($n = 7$) generated a response which had an *I–V* relation with a prominent negative slope region (Fig. 3*C* and *D*), similar to that of light-evoked *I–V* relations recorded in CNQX (see Fig. 2*B* and *D*), or in the light-evoked late *I–V* relation (see Fig. 1*D*). Kainate ($n = 7$) produced a response with a more nearly linear *I–V* relation similar to the light-evoked *I–V* relation seen in AP7 (Fig. 3*B* and *D*). These results reveal that both NMDA and non-NMDA receptors are present on transient amacrine cells, and when combined with the antagonist data demonstrate that the light response is produced by co-activation of NMDA and non-NMDA receptors.

Sustained amacrine cells

The light-evoked I–V relation is nearly linear in sustained amacrine cells

Figure 4*A* shows a typical light response from a sustained amacrine cell. This cell type constituted about 10% of the cell population studied. Its response is characterized by a prominent initial transient followed by a sustained current. Generally, the sustained current amplitude was 10–20% of the amplitude of the initial peak. It was not uncommon to find sustained amacrine cells lacking the initial peak, or to have it disappear during the course of the experiment with no discernible change in the magnitude of the sustained currents (compare Fig. 4*A* and *B*). The response latency was typically 100–150 ms, and activity could persist for up to 700 ms after the light had been extinguished. The time course and shape of the sustained amacrine cell response differed from those of either the on-off amacrine cells, bipolar cells, or the on-off and sustained ganglion cells recorded under the same conditions. Sustained ganglion cells rarely exhibit a prominent peak following the light onset (Mittman *et al.* 1990; D. B. Dixon, unpublished observations). The on-off amacrine and ganglion cell responses are much quieter following the initial transient and exhibit a transient response after the termination of the light stimulus. Bipolar cells have a prominent rebound current at the termination of the light stimulus.

Figure 4(*A–C*) compares the light-evoked currents in a sustained amacrine cell recorded at different holding potentials in control saline (*A*), in AP7 (*B*) or in CNQX (*C*). The average light-evoked *I–V* relation shown in Fig. 4*D* combines data from seven sustained amacrine cells under each of the aforementioned conditions. Again, currents in individual cells were first normalized to their peak value, then all cells were averaged together. The mean current amplitude for all sustained cells was −14·1 pA at −96 mV, ranging between −8·6 and −22·3 pA. Note that for sustained cells, current amplitude was measured by averaging the total current during the response (see below). The *I–V* relation was relatively linear over the range tested (Fig. 4*D*) and reversed between 0 and +10 mV. A small amount of rectification was present at the most negative and positive potentials. The linearity throughout the voltage range from −96 to +24 mV, particularly between −80 and −30 mV, contrasts markedly to the transient amacrine cells which have a negative slope

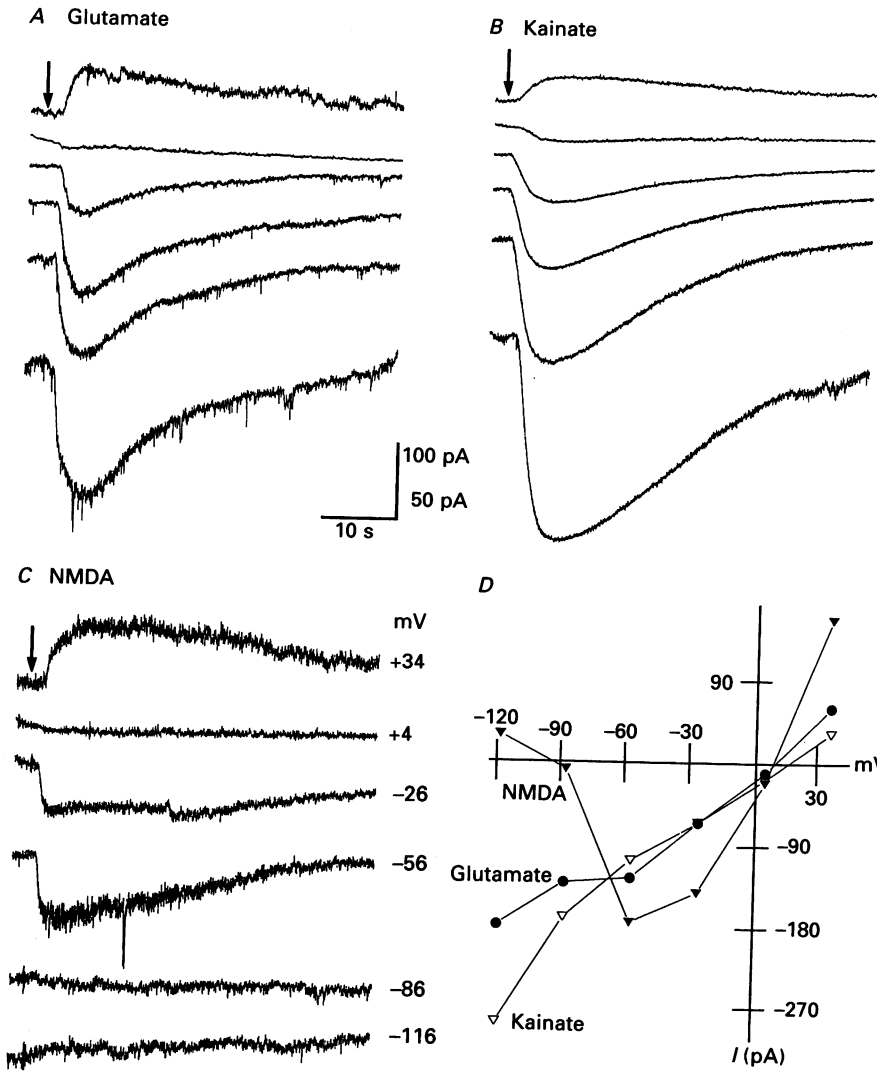

Fig. 3. Effects of glutamate agonists on transient amacrine cells. Agonist concentration in the perfusion pipette was 250 μM; application was for 250 ms at the point indicated by the downward arrow. *A*, responses to glutamate at different clamp potentials. *B*, responses to kainate. *C*, responses to NMDA. *D*, peak *I–V* curves obtained from the cell shown in parts *A* through *C*. The kainate *I–V* relation (▽) was mostly linear; the glutamate *I–V* curve (●) was slightly flattened between −60 and −90 mV; and the NMDA *I–V* curve (▼) was non-linear with a negative slope region. All responses reversed around +10 mV. For kainate and glutamate microperfusion, the superfusion saline contained 20 μM-Cd^{2+}, but contained 5 mM-Mn^{2+} for NMDA microperfusion (see Methods). Clamp potentials are listed to the right of *C*. Scale bar 100 pA for *A* and *B*, 50 pA for *C*. Similar results were obtained in seven cells.

region in their *I–V* relation (see Fig. 1*B*), which we believe corresponds to the activation of NMDA channels. The absence of a flat region in the sustained amacrine cell *I–V* relation suggests that NMDA channels do not participate in their light

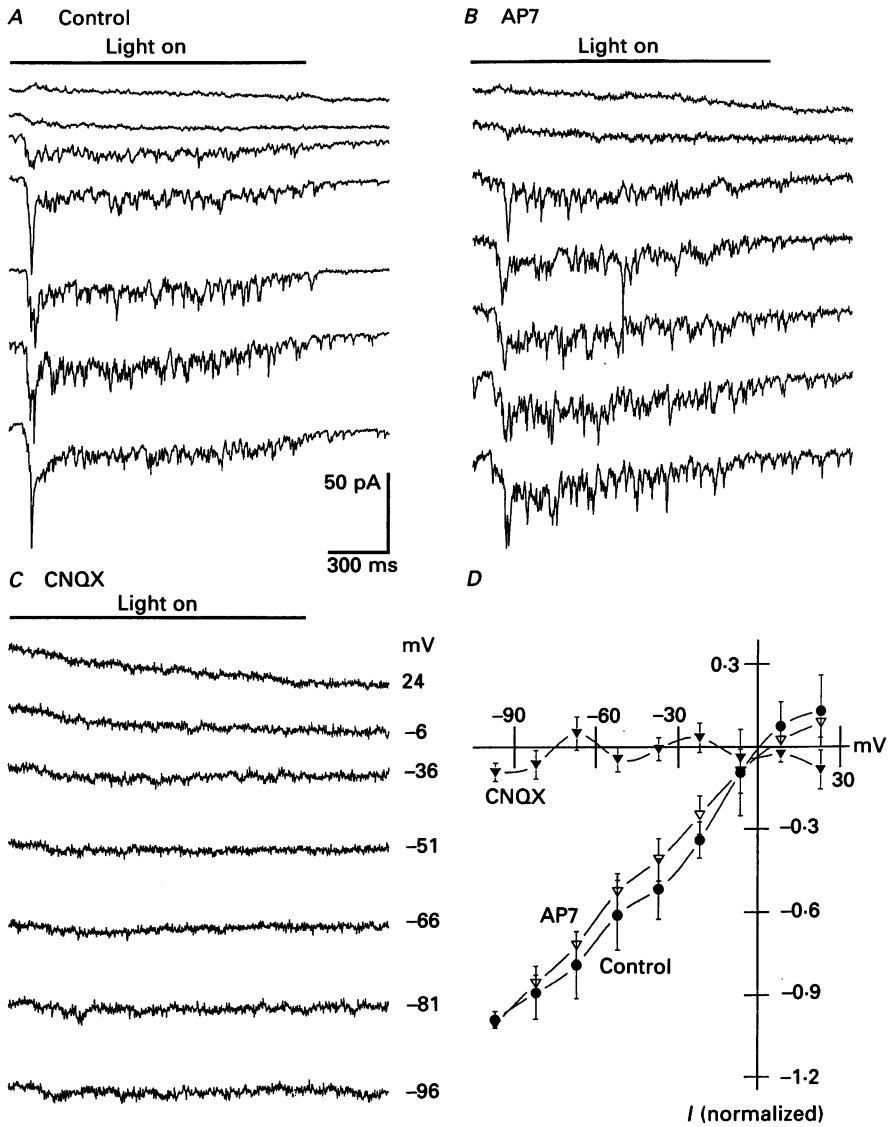

Fig. 4. Effects of glutamate antagonists on light responses of sustained amacrine cells. *A*, responses to light in control saline at holding potentials listed to the right of *C*. *B*, responses of the same cell in AP7 (30 μM). Compared to control, the peak and plateau portions of the response were changed minimally, although there was some reduction in the size of the initial peak. The reduction was not typical of all cells recorded in AP7 and could have resulted from subtle change in the recording conditions. Additionally, the current fluctuations during the response were similar and the reversal potential was unchanged. *C*, responses of the same cell in CNQX (2 μM). The entire light response was eliminated in CNQX. *D*, average, normalized *I–V* relations for seven sustained amacrine cells recorded in control saline (●), AP7 (▽) and CNQX (▼). Error bars indicate S.D.

response. In transient amacrine cells it was possible to distinguish an NMDA component because it was more prolonged. The nature of the sustained amacrine cell response does not permit a similar dissection. However, pharmacological results confirm a lack of an NMDA component in the sustained amacrine cell light response (see below).

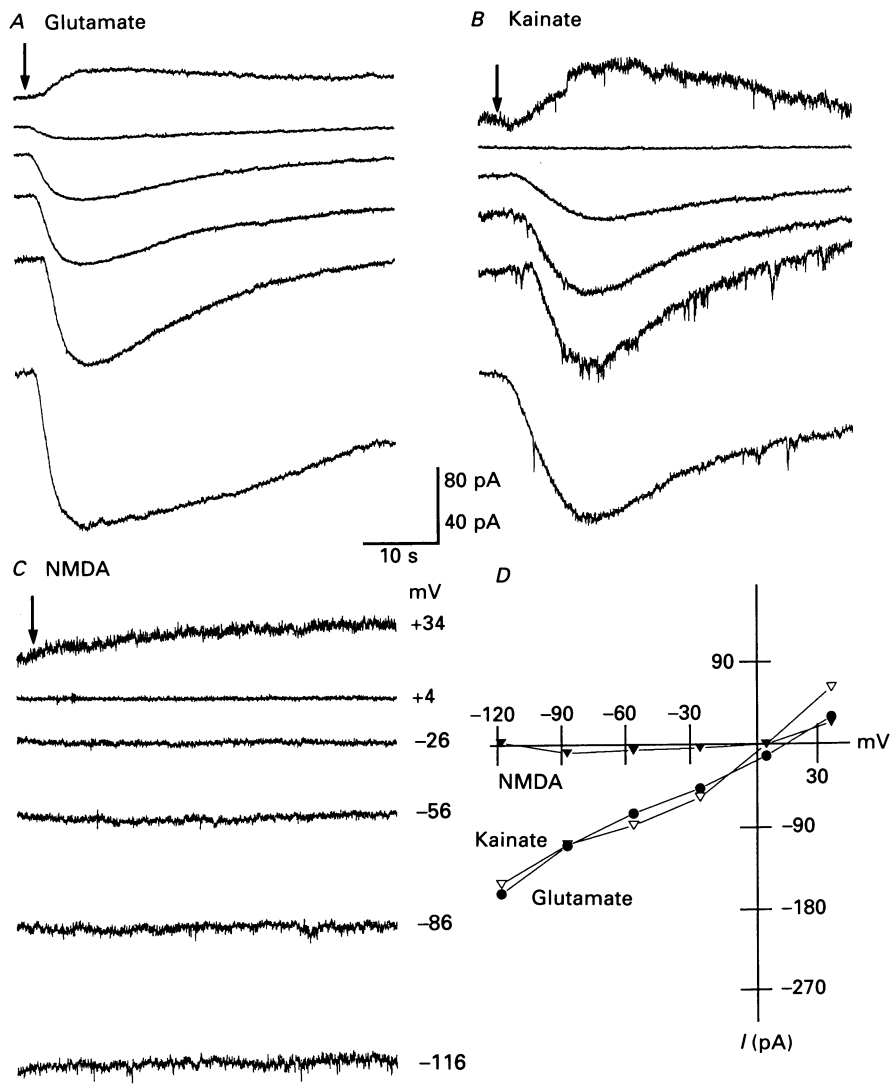

Fig. 5. Effects of glutamate agonists on a sustained amacrine cell. Agonist were applied at the same concentrations and durations as described in Fig. 3. *A*, responses to glutamate at clamp potentials indicated to the right of *C*. *B*, responses to kainate. *C*, responses to NMDA. No response could be obtained with NMDA. *D*, peak *I–V* relations from the cell shown in *A*, *B* and *C* for glutamate (●), kainate (▽) and NMDA (▼). For kainate and glutamate microperfusion the light responses were blocked by 20 μM-Cd^{2+}. For NMDA microperfusion they were blocked with 5 mM-Mn^{2+}. Scale bar 80 pA for *A* and *B*, 40 pA for *C*. Similar results were obtained in three cells.

In 30 μM-AP7, an NMDA antagonist, the light-evoked response and *I–V* relation were unchanged (Fig. 4*B* and *D*). In this particular cell shown in Fig. 4*B*, the initial peak was lost prior to switching to AP7. In 2 μM-CNQX, a non-NMDA antagonist, the entire light response was eliminated suggesting that only non-NMDA receptors are activated during light stimulation is sustained amacrine cells (Fig. 4*C*). We presume that the loss of the entire response in CNQX is not due to blockage of the photoreceptor–bipolar synapses since NMDA-mediated responses in transient amacrine cells and ganglion cells could be recorded at similar concentrations of CNQX.

The light-evoked *I–V* curves for the sustained amacrine cells were measured by calculating the average current for the duration of the light response. Calculating *I–V* curves by either including or excluding the early peak did not alter their linearity or reversal potential.

Sustained amacrine cells respond to non-NMDA agonists but not NMDA agonists

Figure 5 shows an example of a sustained amacrine cell response to glutamate (*A*) or kainate (*B*) in the presence of 20 μM-cadmium. These responses had nearly linear *I–V* relations. In 5 mM-Mn^{2+}, NMDA produced no response in this same amacrine cell (Fig. 5*C*). In contrast, NMDA responses in transient cells were small but reproducible. The lack of NMDA responses in sustained cells suggests the absence of this receptor type; however, since most calcium channel antagonists (used here to block synaptic transmission) can also at least partially block NMDA channels (Mayer & Westbrook, 1987*b*; Mayer, Vyklicky & Westbrook, 1989), we have to interpret this finding somewhat cautiously.

DISCUSSION

Our experiments have demonstrated that both non-NMDA and NMDA receptors are present on transient amacrine cells and that both receptor types provide significant contributions to the light-evoked current. Non-NMDA receptors gate a voltage-independent fast component of the light response whereas the NMDA receptors gate a voltage-dependent slow component. The transient on-off ganglion cells of the salamander retina exhibit similar NMDA and non-NMDA co-activation (Mittman *et al.* 1990). It seems evident that the pathways signalling transient on and off responses rely on the concomitant activation of both non-NMDA and NMDA receptors.

Sustained amacrine cells do not respond to exogenous NMDA and their light responses are not likely to be mediated by NMDA receptors. The sustained light response is comprised of many rapidly occurring, short duration events which sum to generate a sustained current. This current is eliminated by CNQX, consistent with the notion that it is generated by non-NMDA receptors. Similar non-NMDA receptor activity consisting of rapidly occurring brief transients has been found in other systems (Trussell *et al.* 1988; Tang, Dichter & Morad, 1989). Also, Perkel *et al.* (1990) demonstrated that some glutamatergic synaptic inputs to Purkinje cells in the cerebellum are driven solely by non-NMDA receptors.

This singular activation by non-NMDA receptors contrasts markedly with the

dual non-NMDA and NMDA excitatory inputs driving sustained ganglion cells. It seems that the sustained excitatory signals relayed through interneurons in this retina do not rely on NMDA receptors. It will be interesting to see if this finding can be generalized to other species.

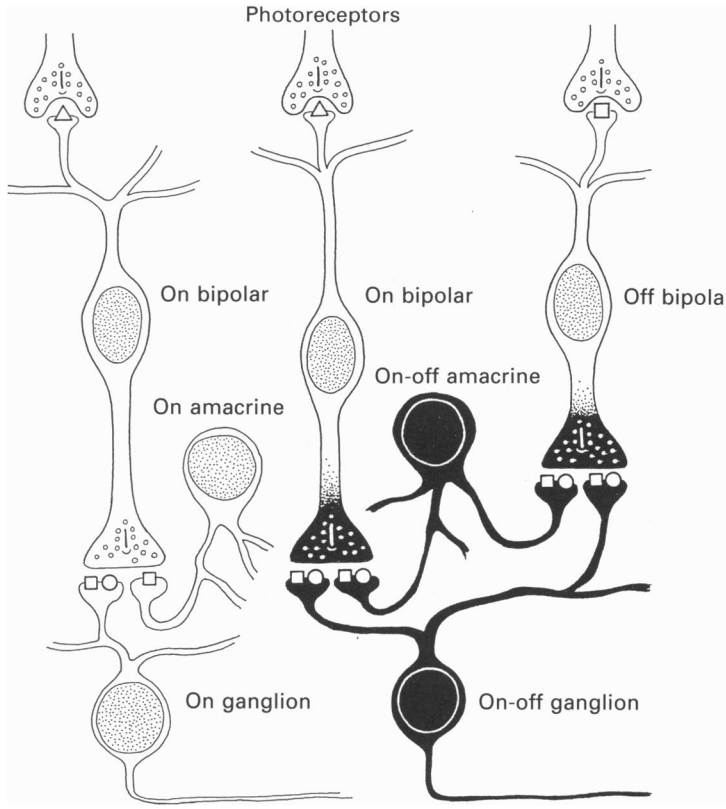

Fig. 6. Schematic representation of the glutamatergic excitatory pathways in the salamander retina. The light-evoked response in transient amacrine cells and in all ganglion cells is mediated by non-NMDA (□) and NMDA (○) co-activation. On sustained ganglion cells also have a mixed non-NMDA and NMDA input, whereas on sustained amacrine cells have exclusively non-NMDA inputs. Bipolar cells inputs are through the non-NMDA and AP4 (△) receptor types.

Figure 6 (modified from Mittman *et al.* 1990) incorporates the findings of this study into a general scheme for glutamatergic excitatory pathways in the retina. Glutamate receptors on bipolar cells are believed to be the non-NMDA type for the off class of bipolar cells and the 2-amino-4-phosphonobutanoate (AP4) type for the on class of bipolar cells (Shiells, Falk & Naghshineh, 1981; Slaughter & Miller, 1983; Nawy & Copenhagen, 1987). Constant light produces sustained responses in photoreceptors and bipolar cells. In the inner retina, some responses are reshaped and become transient in nature, indicated in Fig. 6 by the dark shading. All third order transient cells are excited by both non-NMDA and NMDA receptors. Similarly, on ganglion cells exhibit non-NMDA and NMDA components in their response to light. However,

on amacrine cells lack the NMDA receptor; their light response is made up solely of non-NMDA receptor action. The voltage response of on ganglion cells is marked by repetitive spiking during light stimulation, whereas on amacrine cells fire only a few spikes at light on, remaining depolarized but quiet during the bulk of light stimulation. It is conceivable, but remains to be proven, that the NMDA receptors provide an additional amount of current in the on ganglion cells which boost the membrane potential above threshold for action potentials resulting in an increased excitability. Under constant light conditions, the lack of action potentials in sustained on amacrines cells (resulting presumably from lack of NMDA receptors) could serve to preserve this cells sensitivity to change, its predominant characteristic (Werblin, 1972).

Our data are in contrast to earlier reports (Lukasiewicz & McReynolds, 1985; Coleman & Miller, 1988; Massey & Miller, 1990) suggesting that NMDA receptors contribute little or nothing to the amacrine cell light response. The previous studies did not focus strictly on excitatory inputs and relied on single microelectrode recordings (as opposed to whole-cell voltage-clamp) for voltage control. We found that it was difficult to isolate the excitatory inputs without pharmacologically blocking the inhibitory inputs to these cells.

Could NMDA responses be washed out of sustained cells?

MacDonald, Mody & Salter (1989) reported that NMDA currents in the brain slice preparation ran down with time. Transient amacrine cells and all ganglion cells (Mittman *et al.* 1990) had a large NMDA component in their light response for the duration of the recordings, which lasted up to 90 min. Unless NMDA responses in sustained amacrine cells are particularly vulnerable to wash-out, it is unlikely that an NMDA current run-down contributed to the lack of sustained amacrine cell response to NMDA microperfusion. Furthermore, light *I–V* relations were sampled within the first minute after breaking into the cell, hence they should have shown some indication of an NMDA component if one had been there. From the above data, NMDA receptors do not appear to be present on sustained amacrine cells and do not play a role in the light response (see Fig. 4).

Glutamate is the predominant excitatory transmitter onto amacrine cells

Bipolar cell terminals are immunoreactive to glutamate (Ehinger *et al.* 1988; Marc *et al.* 1990) and on this basis glutamate appears to be the major neurotransmitter to amacrine cells. However, some amacrine cells exhibit acetylcholine- and serotonin-like immunoreactivity (Millar, Winder, Ishimoto & Morgan, 1988; Dann, 1989). Bipolar cells represent the single largest source of presynaptic elements to amacrine cells (Wong-Riley, 1974), but there are amacrine-to-amacrine cell synapses. Since there is no detectable light-evoked activity in the amacrine cells in the presence of 2 μM-CNQX and 30 μM-AP7, it can be concluded that their light-induced excitation is mediated principally through glutamatergic synapses from bipolar cells. Other excitatory transmitters present in the retina might be released during the course of a light response; however, their role is uncertain at the present time.

Conversion of sustained bipolar cell responses to transient amacrine cell responses

Bipolar cells respond to light with a sustained current (Werblin & Dowling, 1969; Nawy & Copenhagen, 1987; data not shown). On-off amacrine cells and on-off ganglion cells respond with short duration current transients at light on and off. These transient, rather than sustained responses are not the result of any voltage- or time-dependent conductances in the membrane of the postsynaptic cells since recordings were performed under voltage clamp. Instead, they must reflect the time course of synaptic excitation. The transience is not due to inhibitory inputs because the responses are recorded in the presence of glycine and $GABA_A$ receptor antagonists. How then is the sustained bipolar cell response converted to a transient response in amacrine and ganglion cells? One possibility to explain this conundrum is that a voltage-dependent calcium current in bipolar cell terminals inactivates, thereby truncating neurotransmitter release (Maguire, Maple, Lukasiewicz & Werblin, 1989). Modification of such a current through $GABA_B$ receptors has been proposed as a potential mechanism for modulating the conversion of the sustained bipolar cell response to a transient format in some amacrine and ganglion cells in the salamander retina (Maguire *et al.* 1989).

For sustained amacrine cells, the mechanism generating the peak and plateau phase of the light response may be similar to the one mentioned above. The rapid reduction of the initial peak response could result from the inactivation of a presynaptic calcium current which shuts off transmitter release; the plateau phase might result from an incomplete inactivation of this calcium current. Alternatively, transmitter release governing the plateau phase of the sustained response could be mediated through a calcium-independent release mechanism. These ideas are left to be explored in future experiments.

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